SR Ca^{2+} stores was also directly measured with chlortetracycline (CTC), a lipophilic, fluorescent, Ca-sensitive dye that can be used to measure stored Ca^{2+} when the Ca^{2+} concentration in the stores exceeds about 10^{-4} M. We observed an ouabain-induced, TG-sensitive increase in CTC fluorescence in the cultured arterial myocytes. We conclude that the Na/Ca exchanger in VSM plays an important role in regulating mobilizable SR Ca^{2+} and in controlling responsiveness to vasoconstrictors.

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THE SARCOPLASMIC RETICULAR CALCIUM PUMP CONTRIBUTES TO Ca^{2+} EXTRUSION FROM VASCULAR SMOOTH MUCSLE

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We have previously reported that the release of Ca^{2+} from the SR by caffeine or ryanodine, or inhibition of SR Ca^{2+} accumulation by thapsigargin increases the steady state $[Ca^{2+}]_i$ in smooth muscle of the isolated rabbit inferior vena cava [1]. The increase in $[Ca^{2+}]_i$ is not accompanied by an increase in divalent cation permeability [2]. In the present report we explore the possibility that interference with SR Ca^{2+} accumulation slows Ca^{2+} extrusion. De-endothelialized rabbit inferior vena cava was loaded with fura-2/AM for the recording of $[Ca^{2+}]_i$ in a Spex spectrofluorimeter. After first raising $[Ca^{2+}]_i$ with a high K^+, high Ca^{2+}_i PSS, the subsequent removal of external Ca^+ resulted in a decline of $[Ca^{2+}]_i$. Comparison of the above types of rates of $[Ca^{2+}]_i$ decline under different experimental conditions showed that prior SR Ca^{2+} depletion by caffeine, ryanodine or thapsigargin caused a slowing of the rate of $[Ca^{2+}]_i$ decline. Control experiments established that these effects did not result from shifts in organellar Ca^{2+} content, but rather were due to inhibition of Ca^{2+} extrusion from smooth muscle cells. Inhibition of Na^+/Ca^{2+}-exchange by removal of external Na^+ slowed down the rate of $[Ca^{2+}]_i$ decline to a similar extent and there were no significant additive effects between external Na^+ depletion and thapsigargin administration. These results lead to the conclusion that SR Ca^{2+} accumulation is a contributory step in Ca^{2+} extrusion from vascular smooth muscle and suggest that the pathway involved consists of Ca^{2+} uptake by the SR pump, vectorial release towards the plasmalemma and Ca^{2+} extrusion coupled to Na^+ influx as proposed in the Superficial Buffer Barrier hypothesis. Calculations show that at least half of all Ca^{2+} extrusion is accomplished via this pathway.

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MYOSIN LIGHT CHAIN KINASE (MLCK) vs. LEIOTONIN

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We isolated a protein factor, leiotonin, which activated the smooth muscle actomyosin (AM) system without phosphorylating myosin light chain (MLC), but it was a proteolytic product. Subsequent effort to pursue the original leiotonin has revealed no parallelism between the actomyosin activation and MLC phosphorylation.

In the meantime, Kuwayama et al. (1988) showed that bovine stomach smooth muscle contained two types of MLCK, 155 and 130 kDa, and the former possessed about ten times stronger AM-activating effect than the latter on the basis of equivalent MLCK activity.

On the other hand, Kobayashi et al. (1992) determined the whole sequence of the 155 kDa component, which as a protein could not be distinguished from MLCK. However, this work unveiled in the N-terminal region the actin binding site which, being absent in the 130 kDa component, seemed crucial for AM activation.

Recently, we have found that wortmannin, a specific MLCK inhibitor, is a more typical agent in removing MLCK activity without reducing AM-activating effect. Beryllium sulfate affects the AM-activating effect more intensely than the MLCK activity. Thus the 155 kDa component exerts its physiological function through the mechanism not directly related to MLCK activity.

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